ELECTROCHEMICAL RADIOIODINATION OF ESTRADIOL

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SUMMARY

An electrochemical iodination procedure was used to synthesize 2-iodoestradiol-^{125}I and 4-iodoestradiol-^{125}I. These agents were prepared in order to be studied as potential tumor-localizing radiopharmaceuticals that might differentiate estrogen-responsive from non-responsive breast tumors. The advantages of the electrochemical iodination procedures, as opposed to other chemical and enzymatic methods of iodination, are the higher percentage of incorporation of iodine, shorter reaction time, selective monoiodination, and a very limited amount of impurities as by-products of the reaction.

Key Words: Electrochemical Iodination, Iodine-125, 2-Iodoestradiol, 4-Iodoestradiol

INTRODUCTION

Recent interest in developing a method for predicting the therapeutic responsiveness of mammary tumors to hormone treatment, based on the binding of estradiol to specific receptors in mammary tissue, has led us to study radioiodinated estradiol as a possible tumor localizing agent. The preliminary results (1) were sufficiently encouraging to pursue this work.

In general, radioiodination can be affected by one of the following techniques: chemical, enzymatic or electrochemical. In our previous work, we had used a chemical method (2) while others had proposed an enzymatic technique reported to yield pure 2-iodoestradiol (3,4). The present report describes a study of an electrochemical method (5) of radioiodination of estradiol.

Iodination of estradiol leads to a mixture of the 2-iodo, the 4-iodo and the 2,4-diiodoestradiols in varying proportion (2). The preliminary biological results attained with this mixture made it desirable to gain more detailed spec-

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ificities of each of these three compounds. A comparative study of radioiodination procedures was undertaken in an attempt to both improve the yields of the iodinated estradiols and vary the proportions of the two monoiodinated isomers, both among themselves and in relation to the diiodinated species.

The chemical method of iodination (2) that we used in our preliminary experiments involved reacting I_2^* in ethanol with estradiol in an ethanol-ammonia solution. A 1:1:1 mixture of the monoiodinated estradiols and 2,4-diiodoestradiol was produced in an overall radiochemical yield of approximately 30%.

In 1971, Matkovics and co-workers (3) reported the enzymatic iodination of estrone and estradiol by hydrogen peroxide and lactoperoxidase using $Na^{131}I$. They reported that only the 2-iodo isomer was formed. Preliminary results in this laboratory confirmed this finding but also suggested lower yields and some other impurities. As the 4-iodo isomer was also desired for comparison of biological specificity, other methods of iodination were required.

A mild and efficient method of electrochemical iodination, using constant current electrolysis, had been developed by Rosa and co-workers (6,7,8) for the labeling of proteins used in radioimmunoassay procedures. In these species iodination would proceed primarily at the tyrosyl residue (8) and was limited to a low level of iodine incorporation (7). Because estradiol also possesses a phenolic function, we decided to develop a modified procedure for the electrochemical radioiodination of estradiol.

MATERIALS

The electrochemical cell used in this work is based on the model of Rosa and co-workers (7), with some modifications, and is made of four components:

1. a platinum vessel, 4 ml in volume, which is the anodic compartment where the labeling occurs.
2. a platinum wire (the cathode) which is separated from the anodic solution by a semi-permeable membrane made of regenerated cellulose.
3. a lucite support holding the cathode and membrane in position.
4. a lead shield.

The power supply was a Hewlett-Packard model 6218A, set at 30 volts and

equipped with a micro-resister to deliver a current of 30 μA. A magnetic stirrer assures mixing of the solution into the anodic compartment. 17β-Estradiol was purchased from Calbiochem; Sodium Iodide (reagent grade) from J.T. Baker Chemical Co.; $Na^{125}I$ (carrier free) from New England Nuclear; Silica gel plates (0.25 mm) from Eastman Kodak; Solvents (reagent grade) from Matheson, Coleman and Bell.

METHODS

1. Electrochemical Iodination

The general method used for labeling of estradiol was: 1 ml of a 0.5 mM solution of estradiol in dioxane and 0.5 ml of a 0.33 mM solution of NaI in 0.04 M phosphate buffer, pH 7.4, labeled with 10 mCi of ^{125}I final specific activity, 400 μCi/μg were added to the anodic compartment of the electrochemical cell. The cathode compartment contained 1 ml of a solution of NaCl (10 mg/ml in a 0.04 M phosphate buffer, pH 7.4). The mixture was electrolyzed at a constant current of 30 μA for 30 minutes. Iodination yields, ranging from 55-60%, were obtained.

2. Purification

Separation and purification of the iodinated estradiols was accomplished by preparative TLC. The iodination mixture was applied to a 0.25 mm thick silica gel plate, previously washed in benzene-methanol (9:1), and developed in dichloromethane (2). The following R_f values were obtained: iodide, 0.0; estradiols, 0.24; 4-iodoestradiol, 0.39; 2-iodoestradiol, 0.50; and 2,4-diiodoestradiol, 0.59. Different radioactive bands, corresponding to unreacted iodine, monoiodinated and diiodinated estradiols, were located by scanning on a radiochromatogram scanner (KESCO Gamma Gram) with a 1" sodium iodide scintillation detector. The relative amounts of each of the radioisomers of iodoestradiol were quantitated automatically during the scanning process by the digital output of the radiochromatogram scanner. The desired bands thus located, representing pure 2-iodo and 4-iodo estradiol, were scraped off and the labeled product was eluted with ethanol.

RESULTS AND DISCUSSION

In order to determine the optimum condition for the iodination of estradiols, the effect of the following variables were determined: a) estradiol concentration, b) reaction time, and c) electrolysis current. In all studies, the concentration of NaI was maintained constant at 0.165 μmoles, to which 10 μCi of ^{125}I was added.

The affects of varying the concentration of estradiol are summarized in Table 1.

Table 1. Distribution of radioiodine between the iodinated estradiols at 3 estradiol concentrations. Current 15 μA.

Estradiol (μmoles)	Percent Incorporation of Iodine		
	Time of Reaction	20 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
0.125	19.0±2.8	24.0±4.2	5.0±1.4
0.25	13.2±1.7	17.3±2.2	1.2±0.4
0.5	20.0±0.8	23.3±2.1	4.4±0.8
		40 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
0.125	-	-	-
0.25	19.6±0.5	21.8±1.1	2.8±1.1
0.5	24.7±1.5	29.9±1.6	5.0±0.9
		60 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
0.125	-	-	-
0.25	22.0±1.2	26.2±2.4	5.8±0.4
0.5	29.3±1.1	33.3±1.7	4.9±0.9

The results represent the averages of 4 or more analyses

At a current 15 μA, the yield in radiolabeled 2- and 4-iodoestradiols was maximal when 0.5 μmole of estradiol was used, and decreased when higher and lower quantities were utilized. In addition, the formation of 2,4-diiodoestradiol-^{125}I, up to 5%, was also observed at the lower concentrations, but was absent when 0.75 and 1.0 μmole of estradiol were used. However, upon increasing the estradiol concentration beyond 0.5 μmole, precipitation and hereterogeneity occurred.

Variations in the electrolysis current produced much more eratic results. Table 2 tabulates the values of 15, 30 and 60 μA after 10, 20 and 30 minutes of electrolysis using 0.5 μmole of estradiol and 0.165 μmole of sodium iodide.

Table 2. Distribution of iodine between the 3 iodinated estradiols at 3 electrolysis currents.

Electrolysis Current (μA)	Percent Incorporation of Iodine		
	Time	10 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
15	16.0±3.2	19.0±1.3	0
30	13.0±1.8	16.7±3.0	1.2±0.5
60	14.7±6.9	18.7±7.1	0
		20 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
15	20.0±0.8	23.3±2.1	4.4±0.8
30	22.2±4.2	27.2±3.0	2.7±2.0
60	16.2±5.8	18.0±5.9	0
		30 minutes	
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
15	23.5±1.7	25.0±1.1	5.3±0.4
30	26.3±3.7	30.5±2.4	4.4±1.8
60	-	-	-

The results represent the averages of 4 or more analyses

Estradiol (μmoles): 0.50

NaI (μmoles): 0.165

No clear trend is evident, notwithstanding a possible maximum at 30 μA.

A systemic study of the reaction time was carried out using an electrolysis current of 30 μA and 0.5 μmole of estradiol. The results are tabulated in Table 3.

Table 3. Distribution of iodine between the 3 iodinated estradiols as a function of time. Reaction condition: 0.5 μmoles of estradiols and 0.165 μmoles of NaI at a constant current of 30 μA.

Reaction Time (minutes)	Percent Incorporation of Iodine into		
	2-iodoestradiol	4-iodoestradiol	2,4-diiodoestradiol
5	8.2±3.0	10.2±3.1	0
10	13.0±1.8	16.7±3.0	1.2±0.5
15	20.0±2.0	23.0±4.4	2.0±1.7
20	22.2±4.2	27.2±3.0	2.7±2.0
25	24.8±2.5	31.0±3.7	2.0±1.3
30	26.3±3.7	30.5±2.4	4.4±1.8
35	23.3±2.7	26.0±2.1	1.9±0.8
40	21.5±1.0	22.2±2.1	2.2±2.0
45	16.6±0.5	20.2±2.7	1.8±1.6
50	15.5±1.9	17.5±1.0	2.0±1.9
60	9.4±2.3	11.0±2.3	0

The results represent the averages of 4 or more analyses

A clear maximum yield of 55-60% is noted at 25-30 minutes of electrolysis.

There is a changing relationship between the electrolysis current and the time required to achieve the maximum level of iodine incorporation. There is a direct relationship at first, but if electrolysis is continued beyond the point

of maximum iodine incorporation, there is a resultant decrease in the percentage of radioiodine incorporation. As the concentration of iodoestradiols produced increases, electrolytic reduction (9) of iodoestradiol may become a competing side reaction, resulting in subsequent deiodination. This will result in an overall decrease in radioiodine incorporation as has been observed in this study.

In the electrochemical iodination of insulin, Pennisi and Rosa (7) reported similar results. They observed that the amount of iodinated insulin, measured at any time after maximum incorporation of iodine was achieved, was always smaller than that expected, based on the amount of iodine discharged. These results were attributed to a lower rate of iodine incorporation.

When the concentration of estradiol is increased from 0.125 μmole to 0.5 μmole at a constant electrolysis current of 15 μA, there is a concomitant increase in the maximum amount of iodine incorporated into 2-iodo and 4-iodo-estradiols over a 60 minute period, and up to 5% of 2,4-diiodoestradiol is formed. When higher concentrations of estradiol are used, i.e. 0.75 μmole at 60 μA and 1.0 μmole at 15 μA, the maximum percentage of iodine incorporation was lower than expected and there was no 2,4-diiodoestradiol formed. The lower yields appear to be related to the poor solubility of estradiol in an aqueous/dioxane solution, while the absence of the diiodinated species can be rationalized by the presence of an excess of free estradiol, which precludes any competing reaction of the monoiodinated products with free iodine.

CONCLUSIONS

The above reported results show that constant current electrolysis can be used efficiently for the iodination of estradiol. The procedure we have described has some practical advantages over the other chemical and enzymatic methods of iodination: 1) the iodination reaction can be controlled carefully; 2) the labeling yield is high; and 3) iodination is limited to the formation of the monoiodinated estradiols, 2-iodo and 4-iodoestradiol, the isomers that have the greatest biological specificity in binding the estrogen receptors.

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